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Munich Biotech AG
Forstenrieder strasse 10
82061 Neuried
ALLEMAGNE

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Method of administering cationic liposomes comprising an active drug

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**Munich
Biotech**

Applicant:

Munich Biotech AG

Forstenrieder Str. 10

D-82061 Neuried

Title:

**Method of administering cationic liposomes comprising an
active drug**

09. Jan. 2004

Description

The use of antimitotic drugs, such as taxanes, as therapeutic agents for human patients suffering from diseases which are connected with enhanced mitosis are well known in the art.

- 5 Paclitaxel has a unique mechanism of action and a broad spectrum of anticancer activity because paclitaxel binds to microtubules and promotes tubulin polymerisation and stabilizes the assembled microtubules. As a result, paclitaxel blocks the cell cycle at prophase resulting in an accumulation of cells in the G2/M phase.
- 10 Unfortunately, paclitaxel has extreme low solubility in water, which makes it difficult to provide a suitable dosage form. Currently, paclitaxel is formulated and administered in a vehicle containing Cremophor EL (a polyethoxylated castor oil) and ethanol in a 50:50 (vol/vol) ratio. This solution is diluted 1:10 in saline before being administered to humans. However, various severe side reactions, such as e.g. hypersensitivity and hypertensive reactions, nephrotoxicity
- 15 and neurotoxicity, have been reported in patients due to Cremophor EL formulation.

- Further, even though paclitaxel (among other antitumor drugs) being a potent, well established standard antitumor drug ((1), (2), (3), (4)), drug-unresponsive tumors and metastases are observed frequently in cancer patients ((5), (6), (7)). Genetically instable, rapidly dividing tumor
- 20 cells gain the capacity to overcome the growth inhibitory effect of a selected anti-cancer drug ((8), (9)). This capacity is usually not limited to a single drug (first line) but extends to other drugs which are used after development of the first resistance. Hence, this phenomenon is called multi drug resistance (MDR). As the number of available and approved anti-neoplastic drugs is very limited for many cancer types, many patients succumb since their cancer tissues
- 25 express MDR. The obvious problem therefore is to find methods and means to kill drug-resistant tumors, especially drug resistant cells, which are already resistant against the respective drug.

- A number of approaches were taken to deal with the above mentioned problems. The
- 30 conventional strategy is to increase doses up to the maximal tolerated dose (MTD) and attempts to eradicate as fast and complete as possible all tumor cells ((10), (11)). It is obvious that this strategy causes severe side effects and can not be extended to longer periods.

to accommodate with drug concentrations at the MTD. The patients become therapy refractory.

The most common solution is to start treatment with a second drug ((5), (2), (3), (12), (13)). In the best case the second line treatment is successful and the patient is cured. A common experience however is that tumors only respond for a certain time leading to a temporary regression of the tumor. After that, tumors become also resistant to the second drug. Continuing with this strategy leads to development of multi drug resistant tumors which are finally refractory to all available anti-cancer drugs ((5), (3), (13)).

Another possibility is to treat patients immediately with a combination of 2 or more drugs ((12), (14), (15), (7), (16), (4)). This strategy can be more successful as it decreases the likelihood for development of a double drug resistance. However this strategy needs to explore time- and cost intensively suitable drug combinations. A second disadvantage is that the side effects also may increase ((14), (7)). The therapeutic window concomitantly becomes small and the toxic effects may overlay the envisioned therapeutic benefit. Also in this case, multi drug resistance may develop and the therapy becomes ineffective ((17), (15), (18), (7)).

The consequence of the negative experiences with such traditional treatment strategies is to develop more and more new drugs to extend the above described treatment options. Obviously, it is a very time and cost intensive race for more potent drugs which eventually will lead in many cases to therapy refractory tumors. In recent years, this recognition has led to a new approach to circumvent tumor resistance. It is based on the assumption that the MDR is caused by overexpression of enzymes which enable cells to expel chemotherapeutic drugs. The most famous member of this category of enzymes is called p-glycoprotein (p-gp). It is located in the cytoplasmic membrane and exports in an ATP-driven way ((19), (20)) compounds like paclitaxel or doxorubicin ((21), (22), (23)). This notion led to the development of p-gp inhibitors which are meant to reverse p-gp mediated drug resistance. Hence the term chemosensitizers was coined for this class of molecules. One of the first examples tested was verapamil. Clinical studies however revealed unsatisfactory results maybe due to low specific activity ((20), (24)). The further research led to a second generation of compounds which again were found not to be clinically applicable ((25), (20)). Today a few substances of the third generation one famous being tariquidar are in clinical testings ((26), (27)). The usefulness and broad applicability of these compounds is however still unclear ((25), (20)). Even though much improved in comparison to first generation chemosensitizers, also third generation compounds cause side effects and may have unforeseen consequences for the whole body. Extensive clinical testing is needed and it is so far uncertain if such approaches can become general practise in the future ((25), (20)).

Different delivery systems have been used to enhance the effect of paclitaxel and/or reduce toxicity. Liposomes are one of many carriers that have been developed to enhance aqueous solubility and thus efficiency, combined with less toxicity:

5 U.S. Pat. No. 5,648,090, U.S. Pat. No. 5,424,073 and U.S. Pat. No. 6,146,659 (Rahman et al.) provide a liposomal encapsulated paclitaxel for a method for treating cancer in mammals. These patents disclose a method of administering to the host a pharmaceutical composition of a therapeutically effective amount of liposomes which include a liposome forming material, cardiolipin, and an agent such as paclitaxel, or an antineoplastic derivative of paclitaxel, or a
10 mixture thereof with a pharmaceutically acceptable excipient. In U.S. Pat. No. 6,146,659 a method of administering a taxane to a patient is provided by administering taxane over a period of less than an hour in an amount from about 75 to 300 mg/m² wherein the taxane is liposomally encapsulated. The liposomes disclosed therein are negatively charged.

15 Since the disclosure of McDonald et al., U.S. Pat. No. 5,837,283, it is known that positively charged liposomes specifically target angiogenic endothelial cells. In order to treat angiogenesis connected with a disease characterized by enhanced angiogenesis, a method of how to administer a taxane encapsulated in a cationic liposome is needed. However, no such method is disclosed so far.

20

Thus, the problem underlying the present invention was to provide a method of administering a cationic liposomal preparation comprising paclitaxel to a subject in need thereof in a therapeutically effective amount without severe side effects.

25 The solution was a method of administering to a human patient in need thereof a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% at a monthly dose of about 0.25 mg up to about 60 mg of paclitaxel / kg body weight of said patient.

30

The advantages of the present invention are as follows:

- selective targeting

increased efficacy

reduced toxicity

improved patient compliance

reduced side effects

- lower side effects compared to traditional chemotherapy or with neutral or anionic liposomes
- reduction of disease related pain
- improvement of quality of life
- 5 - stabilization of body weight during treatment
- synergistic effects with traditional therapy regimes

The present pharmaceutical composition can be administered at a monthly dose of about 0.25 mg up to about 60 mg of liposomal paclitaxel / kg body weight (bw) of a patient, preferably of
10 about 0.5 mg up to about 30 mg of liposomal paclitaxel / kg bw and more preferably of about 1.0 mg up to about 15 mg of liposomal paclitaxel / kg bw.

The dose scheme can range from a plurality of times daily to a plurality of times during a month period, each of said times being separated by an interval of between one day and 3 weeks.

15 The present pharmaceutical composition can be administered at a single unit dose scheme of about 0.01 to 10 mg liposomal paclitaxel per kg body weight. On an average, a human patient has about 70 kg body weight and is about 172 cm tall.

In a preferred embodiment of the present invention about 0.05 to about 5 mg liposomal
20 paclitaxel per kg of body weight is administered at a single unit dose. Preferably, 0.1 to 2.5 mg liposomal paclitaxel per kg of body weight per single unit dose is administered.

The suitable dose of liposomal paclitaxel for application to a human patient is in an amount of about 0.01 to 2.5, preferably 0.02 to 1.0, and more preferably 0.05 to 0.5 mg / kg bw at least
25 once a day; about 0.01 to 5.0, preferably 0.02 to 2.5 and more preferably 0.05 to 1.0 mg / kg bw every other day; about 0.01 to 10, preferably 0.02 to 5.0 and more preferably 0.05 to 2.5 mg / kg bw once a week.

In an even further aspect, the cationic liposomal preparation of the present invention
30 comprises at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% and is useful for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential combination therapy with a jointly effective dose of at least one further active agent and/or heat and/or radiation and/or cryo therapy.

35 In a preferred embodiment the liposomal preparation comprises paclitaxel in an amount of about 0.1 mole% to about 8 mole%, preferably in an amount of about 0.5 mole% to about 5

mole%, more preferably in an amount of about 1 mole% to about 4 mole% and most preferably in an amount of about 2.5 mole% to about 3.5 mole%. The cationic liposomal preparation of the present invention comprises substantially no paclitaxel crystals.

5 The liposomal preparation of the present invention comprises cationic lipids in an amount of about 30 mole% to about 99.9 mole%, preferably from about 40 mole% to about 70 mole% and most preferably from about 45 mole% to about 55 mole% and are characterized by having a positive zeta potential in about 0.05 M KCl solution at about pH 7.5 at room temperature.

10

The preferred cationic lipid of the liposomal preparation is N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammonium methylsulfate (DOTAP). However, other useful lipids for the present invention may include:

DDAB, dimethyldioctadecyl ammonium bromide; 1,2-diacyloxy-3-trimethylammonium
 15 propanes, (including but not limited to: dioleoyl, dimyristoyl, dilauroyl, dipalmitoyl and distearoyl; also two different acyl chain can be linked to the glycerol backbone); N-[1-(2,3-dioleoyloxy)propyl]-N,N-dimethyl amine (DODAP); 1,2-diacyloxy-3-dimethylammonium propanes, (including but not limited to: dioleoyl, dimyristoyl, dilauroyl, dipalmitoyl and distearoyl; also two different acyl chain can be linked to the glycerol backbone); N-[1-(2,3-
 20 dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA); 1,2-dialkyloxy-3-dimethylammonium propanes, (including but not limited to: dioleyl, dimyristyl, dilauryl, dipalmityl and distearyl; also two different alkyl chain can be linked to the glycerol backbone); dioctadecylamidoglycylspermine (DOGS); 3-[N-(N',N'-dimethylaminoethane)carbamoyl]cholesterol (DC-Chol); 2,3-dioleoyloxy-N-(2-
 25 (sperminocarboxamido)-ethyl)-N,N-dimethyl-1-propanaminium trifluoro-acetate (DOSPA); α -alanyl cholesterol; cetyl trimethyl ammonium bromide (CTAB); diC14-amidine; N-*tert*-butyl-N'-tetradecyl-3-tetradecylaminopropionamidine; 14Dea2; N-(alpha-trimethylammonioacetyl)didodecyl-D-glutamate chloride (TMAG); O,O'-ditetradecanoyl-N-(trimethylammonioacetyl)diethanolamine chloride; 1,3-dioleoyloxy-2-(6-carboxy-spermyl)-
 30 propylamide (DOSPER); N,N,N',N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-2,3-dioleoyloxy-1,4-butanedi ammonium iodide; 1-[2-(acyloxy)ethyl]2-alkyl(alkenyl)-3-(2-hydroxyethyl)-imidazolinium chloride derivatives as described by S. Bocini et al. (1995) Biochem. 43:15567-

1,2-dioleoyl-3-dimethyl-hydroxyethyl ammonium bromide (DORI), 1,2-dioleyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DORIE), 1,2-dioleyloxypropyl-3-dimethyl-hydroxypropyl ammonium bromide (DORIE-HP), 1,2-dioleyloxypropyl-3-dimethyl-hydroxybutyl ammonium bromide (DORIE-HB), 1,2-dioleyloxypropyl-3-dimethyl-hydroxypentyl ammonium bromide (DORIE-Hpe), 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), 1,2-dipalmitoyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DPRIE), 1,2-disteryloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DSRIE); cationic esters of acyl carnitines as reported by Santaniello et al. [US5498633]; cationic triesters of phosphatidylcholine, i.e. 1,2-diacyl-sn-glycerol-3-ethylphosphocholines, where the hydrocarbon chains can be saturated or unsaturated and branched or non-branched with a chain length from C₁₂ to C₂₄, the two acyl chains being not necessarily identical.

In a preferred embodiment the liposomal preparation comprises optionally at least one neutral lipid. Neutral lipids are lipids which have a neutral net charge. These can be selected from sterols or lipids such as cholesterol, phospholipids, lysolipids, lysophospholipids, sphingolipids or pegylated lipids with a neutral net charge. Useful neutral lipids thereby include: Phosphatidylserine, phosphatidylglycerol, phosphatidylinositol (not limited to a specific sugar), fatty acids, sterols, e. g. containing a carboxylic acid group, cholesterol, 1,2-diacyl-sn-glycerol-3-phosphoethanolamine, including but not limited to DOPE, 1,2-diacyl-glycerol-3-phosphocholines and sphingomyelin. The fatty acids linked to the glycerol backbone are not limited to a specific length or number of double bonds. Phospholipids may also have two different fatty acids. Preferably the further lipids are in the liquid crystalline state at room temperature and they are miscible (i.e. a uniform phase can be formed and no phase separation or domain formation occurs) with the used cationic lipid, in the ratio as they are applied. In a preferred embodiment the suitable neutral lipid is DOPC.

In a further preferred embodiment the liposomal preparation comprises optionally neutral lipids, preferably DOPC in an amount of about 30 mole% to about 70 mole%, preferably from about 40 mole% to about 60 mole% and more preferably from about 45 mole% to about 55 mole%,

It is a further object of the present invention that the cationic liposome preparation which is used therein can be dehydrated, stored for extended periods of time while dehydrated, and then rehydrated when and where they are to be used, without losing a substantial portion of their contents during the dehydration, storage and rehydration processes. To achieve the latter, one or more protective agents such as cryoprotectants may be present. Thus, preferably the inventive cationic liposome preparation comprises a cryoprotectant wherein the

- 5 In a further preferred embodiment the liposomal preparation comprises trehalose in the range of about 5 % (m/v) to about 15 % (m/v) with respect to the total volume of the preparation.

The formulation of the cationic liposomes of the present invention may vary. In a preferred embodiment the molar ratio is 50:47:3 mole% of DOTAP, DOPC and paclitaxel.

Liposomes of various sizes are useful in the present invention. In a preferred embodiment of the present invention cationic liposomes have an average particle diameter from about 50 to about 500 nm, preferably from about 100 nm to about 300 nm.

- 15 The present liposome compositions can be administered systemically, preferably intravenously.

The cationic liposomes of the present invention may be used to treat any form of a condition associated with increased angiogenesis, such as cancer. The pharmaceutical composition of the present invention is particularly advantageous in treating tumors in human patients such as Head & Neck Cancer, gall bladder and bile duct cancer, stomach cancer, gastrointestinal cancer, Kaposi's sarcoma, urothelial cell carcinoma, thyroid gland carcinoma, testicular carcinoma, vaginal cancer, angiosarcoma, soft tissue sarcoma, mesothelioma, chronic myeloid leukaemia, esophageal cancer, hairy cell leukaemia, kidney cancer, liver cancer, multiple myeloma, neuroblastoma, oral cancer, pancreatic cancer, primary central nervous system lymphoma, skin cancer, small-cell lung cancer, bladder cancer, breast cancer, colorectal cancer, endometrial cancer, leukaemia, lung cancer, lymphoma, melanoma, non-small-cell lung cancer, ovarian cancer, prostate cancer and to childhood cancers such as brain stem glioma, cerebellar astrocytoma, cerebral astrocytoma, ependymoma, Ewing's sarcoma/family of tumors, germ cell tumor, extracranial, hodgkin's disease, leukaemia, acute lymphoblastic, leukaemia, acute myeloid, liver cancer, medulloblastoma, neuroblastoma, non-hodgkin's lymphoma, osteosarcoma, malignant fibrous histiocytoma of bone, retinoblastoma,

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1. The first step in the process is to identify the problem or issue that needs to be addressed. This involves gathering information and understanding the context of the problem.

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1. *What is the purpose of the study?*
 2. *What are the research objectives?*
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 12. *What are the implications?*
 13. *What are the recommendations?*
 14. *What are the future research directions?*
 15. *What are the references?*

Further conditions may be wound healing or an inflammatory disease or a chronic inflammatory disease such as rheumatoid arthritis, dermatitis, endometriosis or psoriasis.

Surprisingly, it was found that active agents loaded into cationic liposomes act directly against drug resistant cells. Cationic liposomes comprising an active agent were known to specifically influence angiogenic endothelial cells only. However, the recent findings define a further target and thus complete the anti-tumor activity of cationic liposomes. This is therefore an additional feature of the present invention.

Thus it is a further object of the present invention to use a cationic liposomal preparation comprising an active agent for the manufacture of a medicament against drug resistant cells. The present invention also provides a method of administering a cationic liposomal preparation comprising an active agent to drug resistant cells of a subject in need thereof in a therapeutically effective amount to affect a disease such as cancer.

In an other aspect, the cationic liposomal preparation of the present invention comprises at least one cationic lipid from about 30 mole% to about 99.9 mole%, an active agent in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% and is useful for manufacturing a pharmaceutical composition for affecting drug resistant cells such that the disease is relieved (causing regression) or eventually cured, inhibited the disease or might have been affected with it.

It is a further surprising finding within the present invention that cationic liposomes comprising an active agent act alone or in combination with at least one other treatment therapy against metastasis.

Thus, it is a further object of the present invention to use a cationic liposomal preparation comprising an active agent for preparing a medicament against metastasis. The present invention also provides a method of administering a cationic liposomal preparation comprising an active agent to a subject in need thereof in a therapeutically effective amount to affect metastasis such as delaying or avoiding a metastatic disease. The term "affecting" as used herein generally means that a desired pharmacologic and/or physiologic effect is obtained such as delaying or avoiding the onset or progression of a disease.

In a preferred embodiment the present invention is used for delaying or avoiding liver metastasis.

In an even further aspect, the cationic liposomal preparation of the present invention comprises at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% and is useful for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential combination therapy with a jointly effective dose of at least one further active agent and/or heat and/or radiation and/or cryo therapy for delaying or avoiding metastasis.

In a further preferred embodiment of the present invention the first active agent loaded into the cationic liposomal preparation can be selected from a cytotoxic or cytostatic substance such as an anti-tumor or an anti-endothelial cell active substance, a chemotherapeutic agent or an immunological active substance. In a more preferred embodiment the active agent is selected from a taxane, a camptothecin, a statin, a depsipeptide, thalidomide, other agents interacting with microtubuli such as discodermolide, laulimalide, isolaulimalide, eleutherobin, Sarcodictyin A and B, and in a most preferred embodiment it is selected from paclitaxel, docetaxel, camptothecin or any derivative thereof.

Thus, in a preferred embodiment of the present invention said liposomal preparation comprises a taxane, preferably paclitaxel or docetaxel or a derivative thereof in an amount of about 0.1 to about 20 mol%, preferably in an amount of about 0.5 mole% to about 10 mole%, more preferably in an amount of about 1 mole% to about 5 mole% and most preferably in an amount of about 2 mole% to about 4 mole%.

Within the present invention the further active agent may be a cytotoxic or cytostatic substance such as an anti-tumor or an anti-endothelial cell active substance, a chemotherapeutic agent, an immunological active substance, a compound that reduces or eliminates hypersensitivity reactions or a chemosensitizer.

In a preferred embodiment, the chemotherapeutic agent is selected from antineoplastic agents such as: antimitotic agents like paclitaxel (Taxol), alkylating agents such as platinum containing compounds like cisplatin, DNA topoisomerase inhibiting agents like camptothecin or doxorubicine, RNA / DNA antimetabolites such as 5-Fluorouracil or gemcitabine and other

reactions. In an even more preferred embodiment the compound is selected from the group comprising Ranitidine, Dexamethasone, Diphenhydramine, Famotidine, Hydrocortisone, Clemastine, Cimetidine, Prednisolone, Prednison, Chlorpheniramine, Chlorphenamine, Dimethindene maleate, Indomethazine and Promethazine or any derivative thereof.

5

In a preferred embodiment the chemosensitizer is selected from the group (but not limited to) comprising cell cycle modulators, substances that revert a drug resistance like verapamil, vasoactive substances like anti-hypertensive drugs, substances that modify the charge-related interaction of cationic liposomes with blood components like protamine.

10

Figure Legends

Figure 1: Tumor size of L3.6pl pancreatic tumors 19 days after start of treatment. Treatment with 10% trehalose, Taxol, MBT-0206, Gemzar (gemcitabine) and the combination of both MBT-0206 and Gemzar started 8 days after tumor cell inoculation. Gemzar was applied i.p. at a dose of 100 mg/kg bw twice a week (Mon, Thu). Taxol and MBT-0206 were applied i.v. on a Mon, Wed, Fri schedule at a paclitaxel dose of 5 mg/kg bw. The combination group received both MBT-0206 and Gemzar with the respective schedule. Tumors were measured by palpation with a calliper on day 23 and 27. Mean \pm SEM; n = 9 per group.

20

Figure 2: Metastases at day 19 after start of treatment. Treatment with 10% trehalose, Taxol, MBT-0206, Gemzar (gemcitabine) and the combination of both MBT-0206 and Gemzar started at day 8 after tumor cell inoculation. Gemzar was applied i.p. at a dose of 100 mg/kg bw twice a week (Mon, Thu). Taxol and MBT-0206 were applied i.v. on a Mon, Wed, Fri schedule at a paclitaxel dose of 5 mg/kg bw. The combination group received both MBT-0206 and Gemzar with the respective schedule, n = 9 per group.

25

Figure 3 - 5: The growth inhibitory assay was performed in 24-well plates with each drug concentration tested in duplicate (n=2 wells). 4×10^4 cells per well were seeded into a 24-well plate and incubated over night. The following day, 10-11 concentrations of the respective drug formulation were added for 72 h to cover the range depicted in the respective graphs. Finally, the cell viability was determined by a standard MTT-assay measuring the activity of mitochondrial dehydrogenases.

30

Figure 6 - 7: The growth inhibitory assay was performed in 24-well plates with each drug concentration tested in duplicate (n=2 wells). 4×10^4 cells per well were seeded into a 24-well plate and incubated over night. The following day, 10-11 concentrations of the respective drug

35

formulation were added for 72 h to cover the range depicted in the respective graphs. Finally, the cell viability was determined by a standard MTT-assay measuring the activity of mitochondrial dehydrogenases.

- 5 **Figure 8:** The growth inhibitory assay was performed in 24-well plates with each drug concentration tested in duplicate (n=2 wells). 4×10^4 cells per well were seeded into a 24-well plate and incubated over night. The following day, 11 concentrations of the respective drug formulation were added for 72 h to cover the range depicted in the respective graph. Finally, the cell viability was determined by a standard MTT-assay measuring the activity of
- 10 mitochondrial dehydrogenases.

The following examples should be illustrative only but are not meant to be limiting to the scope of the invention. Other generic and specific configurations will be apparent to those skilled in the art.

15

Examples

1. Human Therapy Treatment Protocol

- 20 This example is concerned with human treatment protocols using the formulations disclosed. Treatment will be of use preventing and/or treating various human diseases and disorders associated with enhanced angiogenic activity. It is considered to be particularly useful in anti-tumor therapy, for example, in treating patients with solid tumors and hematological malignancies or in therapy against a variety of chronic inflammatory diseases such as
- 25 rheumatoid arthritis or psoriasis.

- A feature of the invention is that several classes of diseases and/or abnormalities are treated without directly treating the tissue involved in the abnormality e.g., by inhibiting angiogenesis the blood supply to a tumor is cut off and the tumor is killed without directly treating the tumor
- 30 cells in any manner. In an other application, drug resistant cells such as drug resistant cancer cells or highly proliferative synovocytes in rheumatoid arthritis can be killed directly.

The various elements of conducting a clinical trial, including patient treatment and monitoring, will be apparent to those skilled in the art.

or radiographic procedures. Such patients would also have no history of cardiac or renal disease and any chemotherapy should be stopped at least 2 weeks before entry into the study.

- 5 Prior to application, the formulation can be reconstituted in an aqueous solution in case the formulation was freeze dried. As outlined above, the required application volume is calculated from the patient's body weight and the dose schedule.

10 The disclosed formulations may be administered over a short infusion time. The infusion given at any dose level should be dependent upon the toxicity achieved after each. Hence, if Grade II toxicity was reached after any single infusion, or at a particular period of time for a steady rate infusion, further doses should be withheld or the steady rate infusion stopped unless toxicity improved. Increasing doses should be administered to groups of patients until approximately 60% of patients showed unacceptable Grade III or IV toxicity in any category.

15 Doses that are 2/3 of this value would be defined as the safe dose.

Physical examination, tumor measurements, and laboratory tests should, of course, be performed before treatment and at intervals of about 3-4 weeks later. Laboratory tests should include complete blood cell counts, serum creatinine, creatine kinase, electrolytes, urea,

20 nitrogen, SGOT, bilirubin, albumin, and total serum protein.

Clinical responses may be defined by acceptable measure or changes in laboratory values e.g. tumor markers. For example, a complete response may be defined by the disappearance of all measurable disease for at least a month. Whereas a partial response may be defined by

25 a 50% or greater reduction.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be

30 apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar

35 substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

The present invention includes a method of delivery of a pharmaceutically effective amount of the inventive formulation of an active agent to a target site such as an angiogenic vascular target site of a subject in need thereof. A "subject in need thereof" refers to a mammal, e. g. a human

The route of administration comprises preferably peritoneal or parenteral administration.

For use with the present invention the "pharmacologically effective amount" of a compound administered to a subject in need thereof will vary depending on a wide range of factors. The amount of the compound will depend upon the size, age, sex, weight, and condition of the patient as well as the potency of the substance being administered. Having indicated that there is considerable variability in terms of dosing, it is believed that those skilled in the art can, using the present disclosure, readily determine appropriate dosing by first administering extremely small amounts and incrementally increasing the dose until the desired results are obtained. Although the amount of the dose will vary greatly based on factors as described above, in general, the present invention makes it possible to administer substantially smaller amounts of any substance as compared with delivery systems which only target the pathologic tissue e. g., target the tumor cells themselves.

2. Mono-therapy protocols

Study No.	Indication
CTLTP01	Prostate Cancer
CTLTP05	Gastro-Intestinal Cancer

CTLTP05 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg (mg liposomal paclitaxel / kg)

Standard formulation liposomal paclitaxel

5 50 mol% DOTAP : 47 mol% DOPC : 3 mol% Paclitaxel

Treatment schedule ongoing studies

	Study No.	Schedule	No. of applications
	CTLTP01	3 times a week	N=3
10	CTLTP05	3 times a week with 3 weeks interval each	N=6

Efficacy

Response will be evaluated according to the WHO or RECIST criteria.

15 **3. Combination therapy protocols**

	Study No.	Indication
	CTLTP04	Lung Cancer
20	CTLTP06	Colorectal or Gastric Cancer

Dosing

	Study No.	Dosages
	CTLTP04	1.5 mg liposomal paclitaxel / kg, 1.5 mg/kg + Carboplatin
25	CTLTP06	0.5 mg liposomal paclitaxel / kg, 1.0 mg/kg, 0.5 - 1.0 mg/kg + 5-FU

Standard formulation liposomal paclitaxel

50 mol% DOTAP : 47 mol% DOPC : 3 mol% Paclitaxel

Treatment schedule ongoing studies

	Study No.	Schedule	No. of applications
30	CTLTP04	once weekly	N=14
	CTLTP06	daily with one week interval each	N=14

Efficacy

35 Response will be evaluated according to the WHO or RECIST criteria.

4. Case report #1

Patient:

- 49 years old patient with large therapy resistant recidivism of a mucoepidermoidal carcinoma of the larynx
- metastases cervical, supraclavicular, axillar, mediastinal and pulmonal
- 5 years after first tumor resection, neck tumor dissection and adjuvant radiotherapy
- after repeated therapy of recidivism with multiple resections, plastic surgery, radiotherapy and chemotherapy

Dosing schedule:

- MBT-0206: 50/47/3 (DOTAP/DOPC/paclitaxel)
- Application of 0.06, 0.25, 0.5 and 1.0 mg liposomal paclitaxel / kg bw, i.v.
- One cycle of 3 times a week (on day 1, 3 and 5)

Results:

- Good tolerance while monitoring cardiovascular, pulmonary and serological parameter during and after infusion
- No signs of acute or chronic toxicity
- Reduction of tumor blood circulation
- Strongly reduced progression of tumor growth during 3 months

5. Case reports #2 and #3

One patient with liver cell carcinoma, who had disease progression after multiple chemotherapies, has been treated with MBT-0206 .

Lyophilized MBT-0206 has been reconstituted with water for injection and a total infusion volume of 300-400 ml MBT-0206 (equivalent to a dose of 1.0 mg liposomal paclitaxel / kg body weight BW) has been administered by central or peripheral intravenous infusion over a period of 2-3h. The infusion rate has been increased slowly up to a maximum speed of 0.5

BW) and then a consolidation dose of 19 times 1.0 mg liposomal paclitaxel / kg BW. This treatment is after 22 weekly administrations still ongoing and up to now no adverse drug reactions have been reported. Besides the favourable safety profile the last evaluation of tumor size, which has been performed by CT-Scan of the liver, showed stable disease.

5

In another case a prostate cancer patient who became refractory to hormone therapy, has been treated with 1.0 mg liposomal paclitaxel / kg BW, 3 times weekly every third day under the same conditions of preparation and administration as described above. The premedication contained dexamethasone and antihistamines. The accumulated dose of liposomal paclitaxel for this patient in 7 days was 3.0 mg liposomal paclitaxel / kg BW.

10

6. Low dosing schedule with liposomal paclitaxel

15 Immortalised endothelial cells (EA.hy926) are seeded into 24-well plates (4×10^4 cells per well) and grown over night. The following day, 9 wells are treated for 1 h with the low dose of 51.2 ng/ml paclitaxel (60 nM) formulated as MBT-0206. In addition, 3 wells per formulation are treated with the high dose of 153.7 ng/ml (180 nM) paclitaxel formulated as MBT-0206 for 1 h and 3 wells remain untreated. Approximately 24 h later, 6 of the 9 low dose-treated wells are again treated with the same low doses of MBT-0206 for 1 h (i.e. 2x treatment groups). Again 24 h later, 3 of these 6 two times -treated wells are treated for the third time with 51.2 ng/ml paclitaxel formulated as MBT-0206 for 1h (3 x treatment groups). Approximately 96 h after this third treatment, the cell viability of all wells is quantitated. For this purpose, an assay which measures the activity of mitochondrial dehydrogenases using the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromid (MTT) is applied according to standard protocols (e.g. (28) with slight modifications).

20

The results demonstrate that the viability of cells treated for 3 times with a low dose of MBT-0206 is at least as strongly reduced as the viability of cells treated only one time with a high dose. Cells treated one time or two times with the low dose of MBT-0206 exhibit a somewhat increased viability which is however reduced in comparison to untreated cells.

25

30

Conclusion

Treatments with high doses of MBT-0206 can be replaced by using low doses at a higher frequency. There is a correlation between treatment density (no. of treatments per week) and treatment efficacy. Three weekly treatments with low doses were superior to 1 or 2 weekly treatments. This optimised dosing regimen potentially reduces toxic side effects caused by high dose treatments.

35

7. Anti-tumor efficacy of MBT-0206 in combination with gemcitabine (50 mg/kg) in L3.6pl pancreatic tumors

5

Animal model

Species: male Balb/c nu/nu mice

Tumor model: Solid orthotopic L3.6pl pancreas tumor (highly metastatic; Bruns CJ, Harbison MT, Kuniyasu H, Eue I, Fidler IJ. In vivo selection and characterization of metastatic variants from human pancreatic adenocarcinoma by using orthotopic implantation in nude mice. Neoplasia 1999; 1(1):50-62.)

Supplier: Charles River France

Treatment

15 Treatment start: Day 7 after tumor inoculation

Last treatment: Day 26 after tumor inoculation

Dose: MBT-0206 with 5 mg paclitaxel / kg bw per application

Taxol at 5 mg paclitaxel / kg bw per application

20 Gemcitabine (Gemzar) at 50 mg / kg per application

Schedule: MBT-0206, Taxol, trehalose: d 7, 10, 12, 14, 17, 19, 21, 24, 26

Gemcitabine: d 7, 11, 14, 18, 21, 25

Combination: combined mono treatments

25

Application route: MBT-0206, Taxol, trehalose: i. v. bolus into tail vein

Gemcitabine: i. p. bolus

Groups (n = 9; n = 2-5 at day 26, 28)	Treatment
Trehalose, Taxol, MBT-0206	d 7, 10, 12, 14, 17, 19, 21, 24, 26
Gemcitabine [50 mg/kg]	d 7, 11, 14, 18, 21, 25

STUDIED BY: [illegible]

DATE: 12-11-2003 TIME: 21:01:21

Monitored parameters

Tumor volume palpated at day 10, 12, 14, 17, 19, 21, 24 after inoculation and at day 26, 28
5 after harvesting

Body weight from day 12 before each measurement of tumor volume

Necropsy after harvest at day 26 and 28.

10 ***Results***

No effect of any treatment could be observed by palpation three days after begin of treatment. Strong anti-tumor effect was observed after one week and at day 24 by palpation, with the following ranking in efficacy: Gemzar-50 = Taxol < MBT-0206 < MBT-0206 + Gemzar.
15 However, after harvest at day 26, the measured tumor volumes of all groups were clearly lower compared to day 24. This difference in size is most likely due to imprecise palpation before harvest. At day 24 tumor size is reduced to ~ 30% by the mono treatments compared to the control group (n =2). The combination of MBT-0206 + Gemzar resulted in the strongest reduction of the tumor size to 13 %, which was significantly ($p < 0.05$) more effective
20 compared to Taxol, Gemzar and MBT-0206 alone. At day 28 the control group (n =2) is not shown, because one of the two tumors was extremely small compared all other tumors (day24, 26, 28) and thus considered as not representative. The tumors after the mono treatments showed a weak increase in tumor size compared to day 26 (Taxol: 536 mm³; MBT-0206: 392 mm³; Gemzar: 398 mm³), whereas the combination therapy led to a slight
25 tumor regression between day 26 and 28 to 88 mm³.

These data show a strong anti-tumor efficacy of Taxol, Gemzar and MBT-0206 in this model. The anti-tumor action of MBT-0206 is slightly stronger than Taxol but similar to Gemzar. The combination of MBT-0206 and Gemzar shows an impressive anti-tumor efficacy.
The mortality was low before day 21: only 1 control and 1 MBT-0206 animal died. At day 24
30 the mortality increased for unknown reason: 3 MBT-0206, 1 Gemzar and 4 control animals were dead at day 24.

The body weight was not effected by either treatment, only the weight of control tumors decreased by 18 % during the last week.

35

8. Anti-tumor efficacy of MBT-0206 in combination with gemcitabine (100 mg/kg) in L3.6pl pancreatic tumors

Animal model

Species: male Bald/c nu/nu mice

Tumor model: Solid orthotopic L3.6pl pancreas tumor (highly metastatic)

5 Supplier: Charles River France

Treatment

Treatment start: Day 8 after tumor inoculation (01.05.03)

Last treatment: Day 26 after tumor inoculation (19.05.03)

10

Schedule: MBT-0206, Taxol, trehalose: d 9, 12, 14, 16, 19, 21, 23, 26
 Gemcitabine: d 8, 12, 15, 19, 22, 26
 Combination: combined mono treatments

15

Groups (n = 9)	Treatment
Trehalose, Taxol, MBT-0206	d 9, 12, 14, 16, 19, 21, 23, 26
Gemcitabine [100 mg/kg]	d 8, 12, 15, 19, 22, 26
MBT-0206 [5 mg /kg bw] + Gemcitabine [100 mg/kg]	d 9, 12, 14, 16, 19, 21, 23, 26 d 8, 12, 15, 19, 22, 26

Monitored parameters

25

Tumor volume palpated at day 23, 26 after inoculation and after harvesting

Body weight from day 1, 7, 12, 16, 19, 21, 23, 27

Necropsy after harvest at day 27

30

Results (see figures 1 and 2)

After intratumoral injection of MBT-0206, treatment was observed during 10 days after tumor inoculation. Tumor volume was measured at day 23, 26 after inoculation and after harvesting. Body weight was measured from day 1, 7, 12, 16, 19, 21, 23, 27. Necropsy was performed at day 27.

treatment reduced the final tumor volume to 68%, which was not significant. Interestingly, the efficacy of MBT-0206 and the combination therapy were more pronounced at day 23. Responsible for that might be the extended therapeutic interval during the weekend between day 23 and 27. These data reveal a clear anti-tumor efficacy of Taxol, Gemzar and MBT-0206 in this model. The anti-tumor action of MBT-0206 is slightly stronger than Taxol but similar to Gemzar. Both MBT-0206 and the combination with Gemzar inhibited the formation of metastases (fig. 2). Liver metastases were absent only in the these two groups. Additionally, only in the combination group the lymph node metastases were rare. The data revealed that the combination of MBT-0206 and Gemzar enhances the anti-tumor efficacy of either mono-therapy.

The mortality was slightly increased in the treatment groups, particularly during combinatory treatment (4 mice died). No control animal died before harvest.

The body weight of control mice decreased by 18% during the last 11 days. During this period a transient decrease was also observed for the treated mice leading to a weight loss of 2% for Taxol, 12% for MBT-0206, 19% for the combination and 22% for Gemzar.

9. Killing of paclitaxel resistant cells (e.g. tumor cell lines)

To demonstrate the potential of MBT-0206 to directly kill tumors expressing (multi) drug resistance, two highly paclitaxel resistant mammalian tumor cell lines were investigated in vitro. These cell lines were selected by stepwise increasing the concentration of Taxol® in the culture medium. Both cell lines have developed a high resistance level which is reflected by concentrations for 50 % growth inhibition (IC50 value) for Taxol® around 1 or 5 µM (867 or 5000 ng/ml). In both instances, MBT-0206 is clearly superior to Taxol® in killing drug resistant tumor cells. In contrast, in drug-sensitive or low-resistant cell lines, MBT-0206 has a more or less identical killing potential to Taxol®.

MBT-0206 and the human uterus sarcoma derived cell line Mes-SA and its derivative lines

The highly paclitaxel resistant derivative cell line Mes-SA/Dx-5_{MBT} was selected with increasing Taxol® concentrations from the commercially available line Mes-SA/Dx-5 (ATCC, (29)). As shown in Fig. 3, it is highly resistant to paclitaxel indicated by the IC50 value of 867 ng/ml. Surprisingly, it is found that MBT-0206 is killing this cell line much more effectively, mirrored by the approximately 20-fold lower IC50 value. The commercially available line Mes-SA/Dx-5 which was selected from the parental line Mes-SA with doxorubicin ((29)) expresses a low

level of cross resistance for paclitaxel (compare Figs. 3 - 5). The IC₅₀ value for Taxol[®] is approximately 7-fold lower than in Mes-SA/Dx-5_{MBT}. Concomitantly, there is only a slight tendency of higher killing potential of MBT-0206 compared to Taxol[®] in this cell line (Fig. 4). The parental line Mes-SA is highly sensitive for Taxol[®] indicated by the low IC₅₀ value of 5.5 ng/ml (Fig. 5). Against this drug-sensitive line, MBT-0206 has the same killing potential as Taxol[®]. This is also true for all other paclitaxel-sensitive lines investigated so far. As example for this notion the results of treatments with MBT-0206 and Taxol[®] of the immortalised endothelial line EA.hy926 are shown in Fig. 8.

10 ***MBT-0206 and the murine colon carcinoma derived cell line Colon-26***

In a similar way to Mes-SA/Dx-5_{MBT}, a highly paclitaxel resistant derivative line of the murine colon carcinoma line Colon-26 (Cell lines Service, Heidelberg) was established and called Colon-26_{MBT}. The IC₅₀ value for Taxol[®] is approximately 5 µg/ml (Fig. 6). Again as in Mes-SA/Dx-5_{MBT}, MBT-0206 had a clearly higher potential to inhibit the growth of this cell line. In this cell line, the IC₅₀ values differ by a factor of 3. In line with the result shown for Mes-SA and EA.hy926 cells, the parental drug-sensitive line Colon-26 is equally sensitive for MBT-0206 and Taxol[®] (Fig. 7).

20 ***Conclusion***

In highly paclitaxel-resistant cell lines, MBT-0206 has a significantly higher killing potential as Taxol[®]. In paclitaxel-sensitive lines, both paclitaxel formulations have a comparable efficacy. MBT-0206 may therefore be able to kill also (multi) drug resistant tumors directly in vitro and in vivo. It may be therefore a new approach to treat human tumors (or other diseases) which become unresponsive for paclitaxel.

10. Therapy of recurrent head and neck squamous-cell carcinoma

30 Evaluation of safety of paclitaxel loaded cationic liposomes (MBT-0206) in patients with recurrent, therapy refractory head and neck squamous-cell carcinoma.

- Karnofsky-performance index > 60 %
- Life expectancy > 4 months

Drug Administration

5

- Paclitaxel encapsulated in cationic liposomes was administered in 2 doses: 0.55 mg. Paclitaxel/kg and 1.10 mg Paclitaxel/kg
- After a screening period, the drug was intravenously injected on day 1, 3 and 5

10

Conclusions

During and after injection no signs of acute or chronic toxicity were observed: Vital and lab safety parameters remained nearly constant.

15

The results indicate that the dose and schedule suggested from preclinical toxicology studies was well tolerated by the patients.

Cationic liposomes selectively targeted endothelium of human head and neck squamous cell carcinoma. Laser Doppler flowmetry confirmed the antivasular mechanism of action of the therapy.

20

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09. Jan. 2004

Claims

1. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for administering to a human patient in need thereof at a monthly dose of about 0.25 mg up to about 60 mg of paclitaxel / kg body weight of said patient.
2. The use of claim 1, wherein said monthly dose is about 0.5 mg up to about 30 mg paclitaxel / kg body weight.
3. The use of claim 1 or 2, wherein said monthly dose is about 1.0 mg up to about 15 mg paclitaxel / kg body weight.
4. The use of any one of the claims 1 to 3, wherein administering said cationic liposomal preparation is at least once a time daily.
5. The use of any one of the claims 1 to 4, wherein administering said cationic liposomal preparation is a plurality of times during a month period, each of said times being separated by an interval of between one day and 3 weeks.
6. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, paclitaxel in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential combination therapy with a jointly effective dose of at least one further active agent and/or heat and/or radiation and/or cryo therapy.
7. The use of any one of the claims 1 to 6, wherein said cationic liposomal preparation comprises paclitaxel in an amount of at least about 2 mole% to about 8 mole%.
8. The use of any one of the claims 1 to 7, wherein said cationic liposomal preparation comprises paclitaxel in an amount of about 2,5 mole% to about 3,5 mole%.
9. The use of any one of the claims 1 to 8, wherein said cationic liposomal preparation comprises 50:47:3 mole% of DOTAP, DOPC and paclitaxel.
10. The use of any one of the claims 1 to 9, wherein said cationic liposomal preparation comprises substantially no paclitaxel crystals.
11. The use of any one of the claims 1 to 10 for treating an angiogenesis-associated condition.
12. The use of claim 11 for treating wound healing, cancer, an inflammatory disease or a chronic inflammatory disease such as rheumatoid arthritis, dermatitis, psoriasis or endometriosis.

13. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, an active agent in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition against drug resistant cells.
- 5 14. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, an active agent in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition against metastasis.
- 10 15. The use of claim 14 for manufacturing a pharmaceutical composition against liver metastasis.
16. Use of a cationic liposomal preparation comprising at least one cationic lipid from about 30 mole% to about 99.9 mole%, an active agent in an amount of at least about 0.1 mole% and at least one neutral lipid from about 0 mole % to about 70 mole% for manufacturing a pharmaceutical composition for simultaneous, separate, or sequential
- 15 combination therapy with a jointly effective dose of at least one further active agent and/or heat and/or radiation and/or cryo therapy against metastasis.
17. The use of any one of the claims 13 to 16, wherein said active agent is selected from a cytotoxic or cytostatic substance such as an anti-tumor or an anti-endothelial cell active substance, a chemotherapeutic agent or an immunological active substance.
- 20 18. The use of claim 17, wherein said first active agent is selected from a taxane, a camptothecin, a statin, a depsipeptide, thalidomide, other agents interacting with microtubuli such as discodermolide, laulimalide, isolaulimalide, eleutherobin, Sarcodictyin A and B, and in a most preferred embodiment it is selected from paclitaxel, docetaxel, camptothecin or any derivative thereof.
- 25 19. The use of claim 6 or 16, wherein said further active agent is an anti-endothelial cell active substance, an anti-tumor active substance, a chemotherapeutic agent, an immunological active substance, a compound that reduces or eliminates hypersensitivity reactions or a chemosensitizer.
- 30 20. The use of claim 19, wherein said chemotherapeutic agent is selected from antineoplastic agents such as: antimitotic agents like paclitaxel (Taxol), alkylating agents such as platinum containing compounds like cisplatin, DNA topoisomerase inhibiting agents like camptothecin or doxorubicine, FFA / DNA antimetabolites such as 5-fluorouracil or methotrexate and other compounds having structure according to the formula (I) or (II):

22. The use of claim 21, wherein said compound is selected from the group comprising Ranitidine, Dexamethasone, Diphenhydramine, Famotidine, Hydrocortisone, Clemastine, Cimetidine, Prednisolone, Chlorpheniramine, Chlorphenamine, Dimethindene maleate, and Promethazine.
- 5 23. The use of claim 19, wherein said chemosensitizer is selected from the group comprising cell cycle modulators, substances that revert a drug resistance like verapamil, vasoactive substances like anti-hypertensive drugs, substances that modify interaction of cationic liposomes with blood components like protamine.
- 10 24. The use of any one of the claims 1 to 23, wherein said cationic liposomal preparation comprises liposomes having an average particle diameter from about 50 nm to about 500 nm, preferably about 100 nm to about 300 nm
25. The use of any one of the claims 1 to 24, wherein said cationic liposomal preparation is administered systemically, preferably intravenously.

Abstract

09. Jan. 2004

The present invention relates to the use of pharmaceutical preparations comprising paclitaxel for administration to a human patient in need thereof.

Figure 1

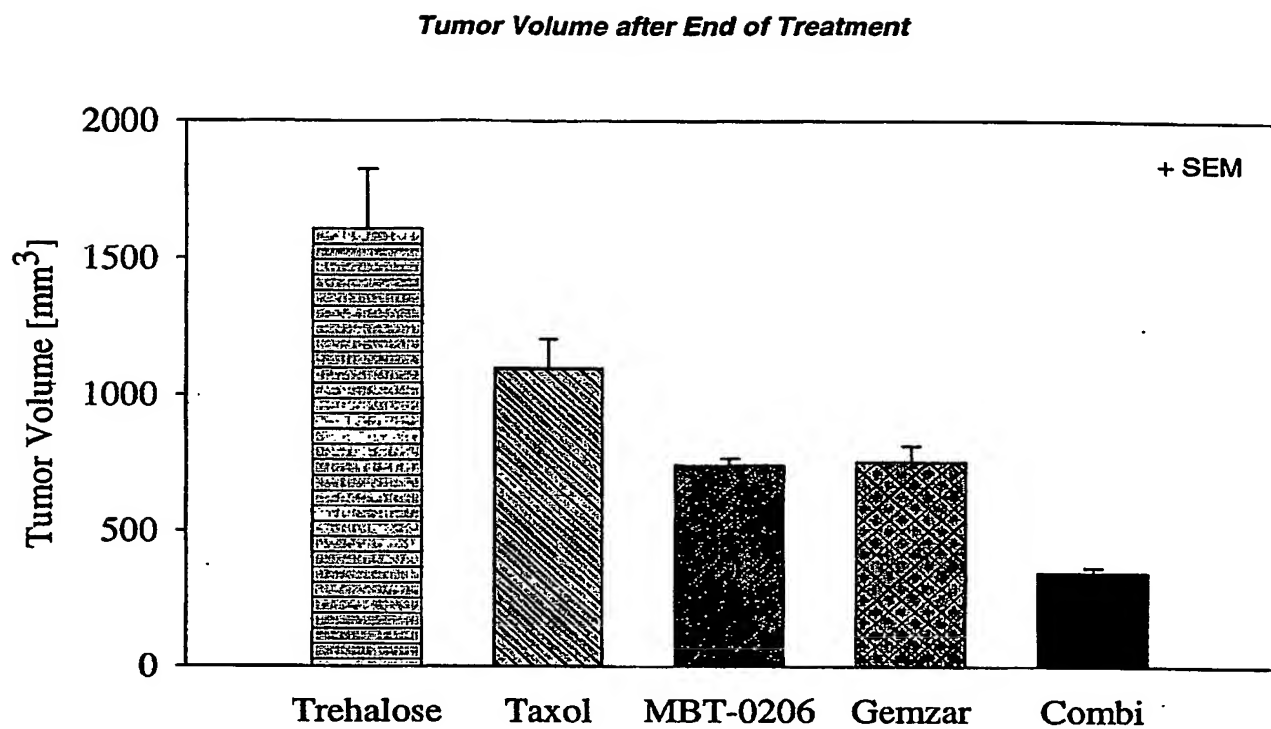


Figure 2

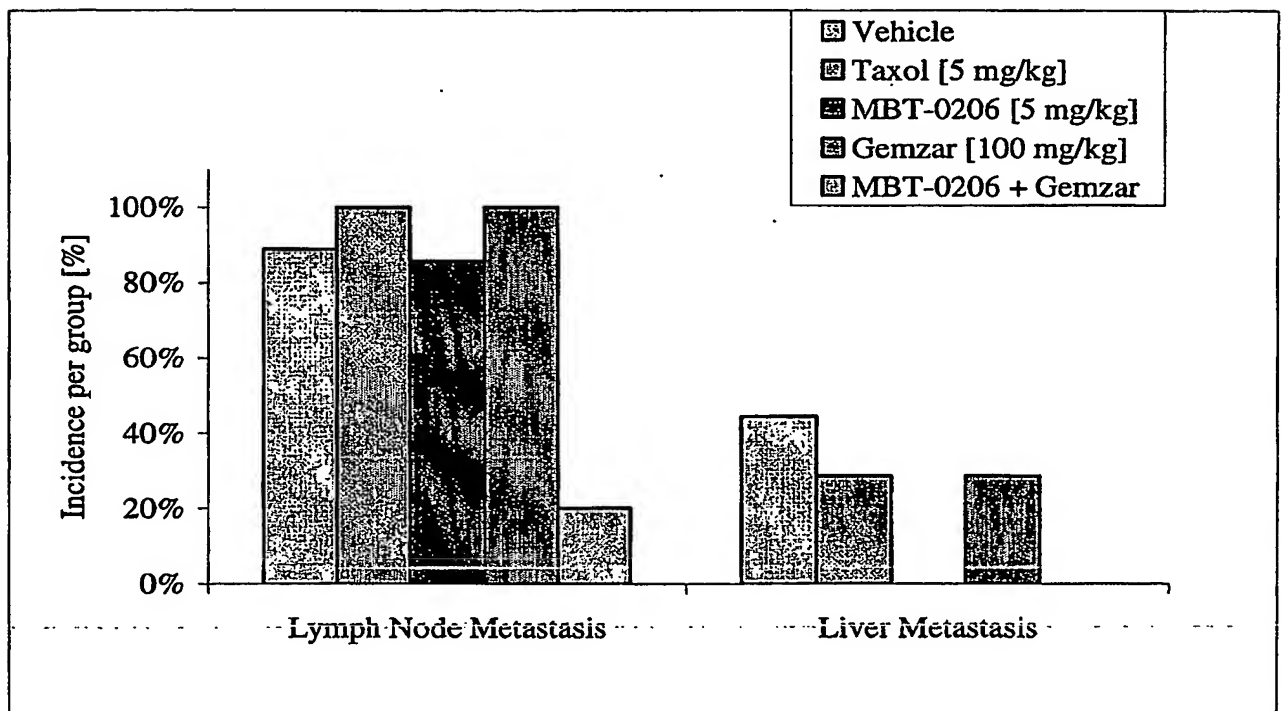
Metastases after therapy

Figure 3

Inhibitory Potential of MBT-0206 and Taxol[®] against the highly drug-resistant uterus sarcoma line Mes-SA/Dx-5_{MBT}

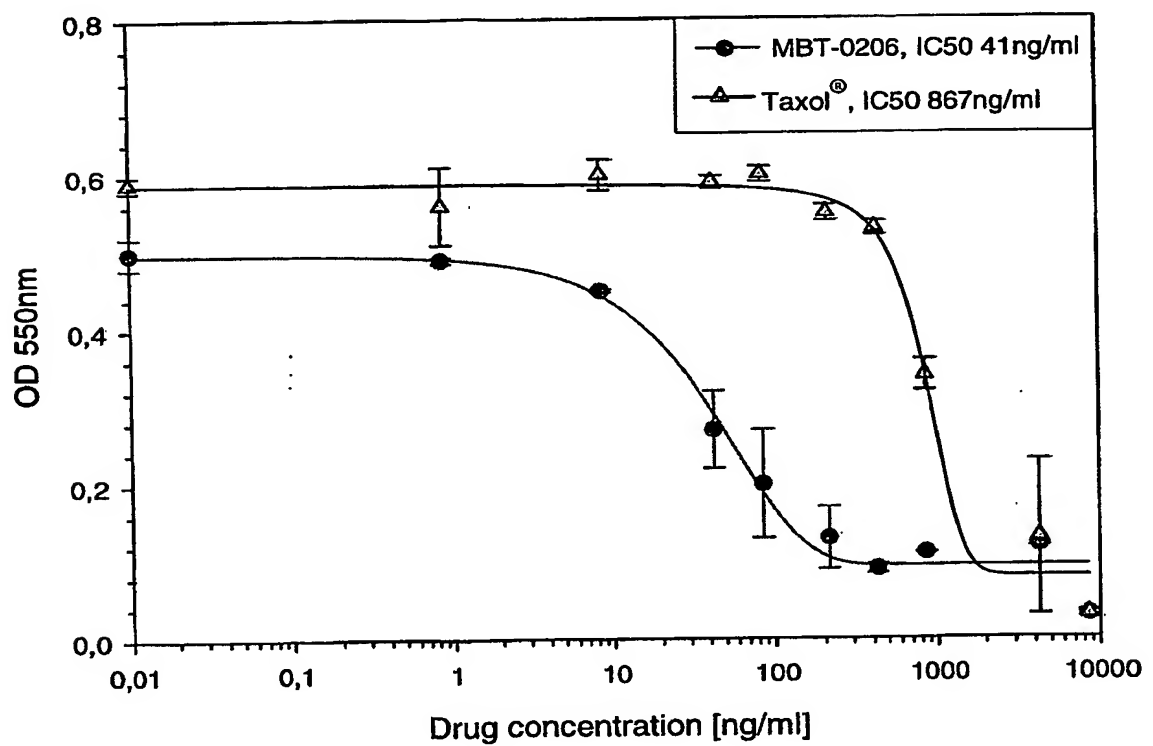


Figure 4

Inhibitory Potential of MBT-0206 and Taxol[®] against the moderately drug-resistant uterus sarcoma line Mes-SA/Dx-5

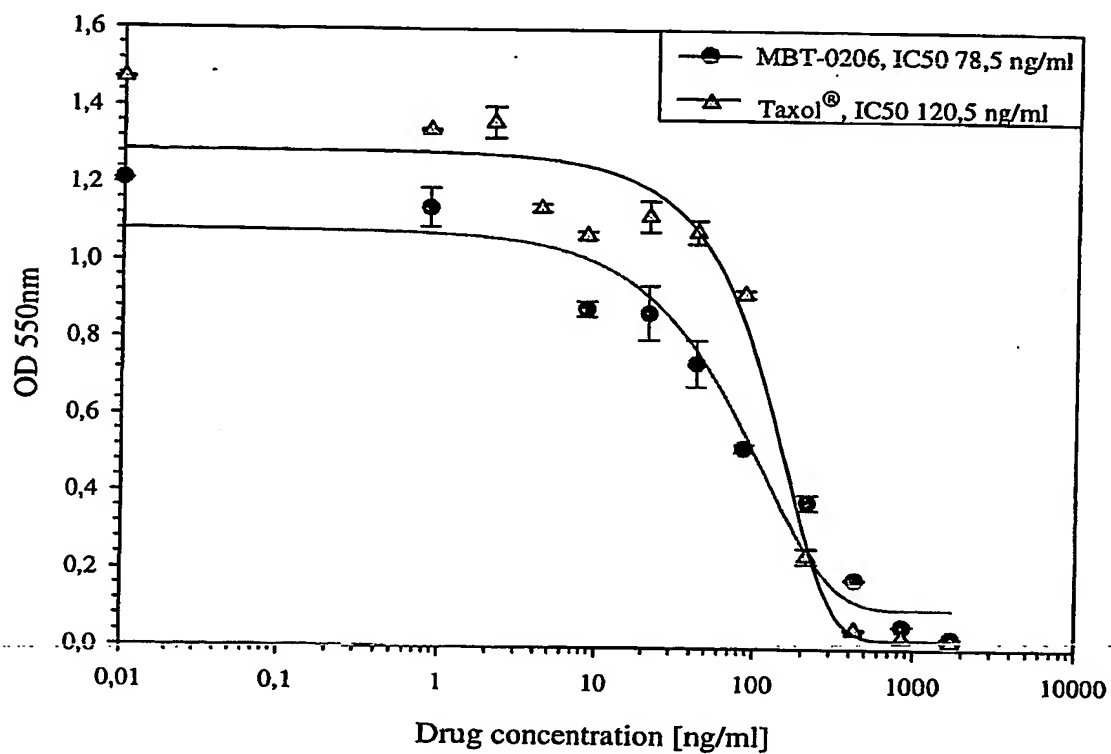


Figure 5

Inhibitory Potential of MBT-0206 and Taxol[®] against the drug-sensitive human uterus sarcoma line Mes-SA

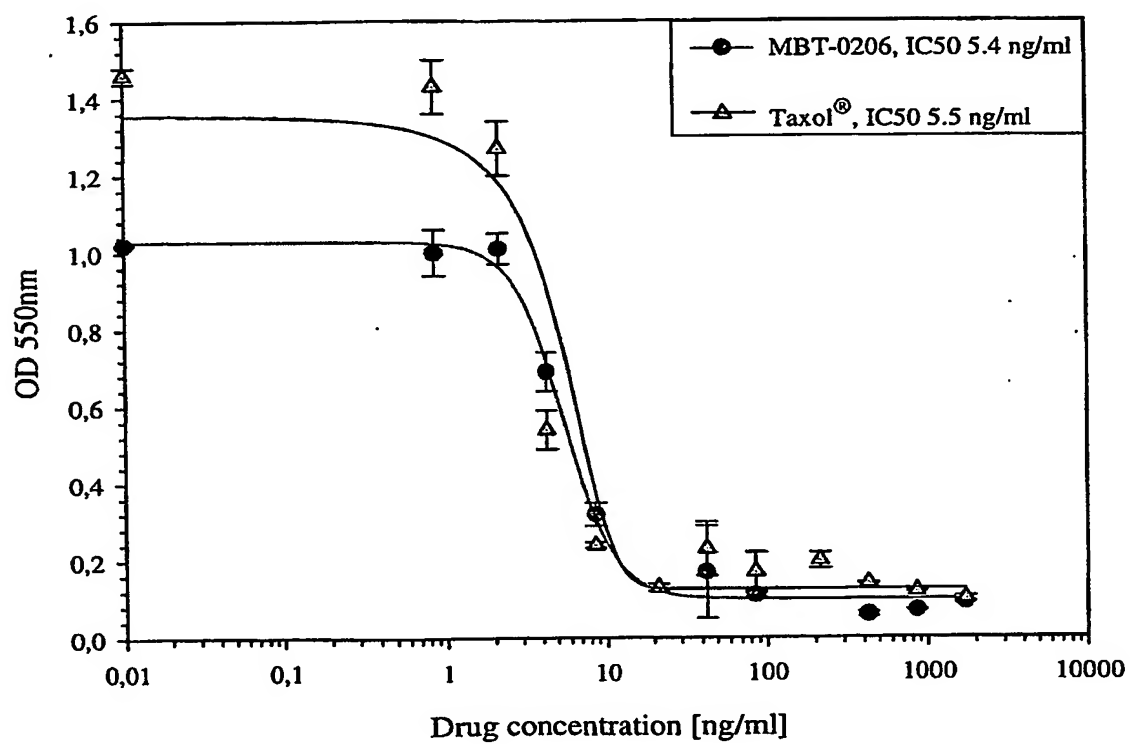


Figure 6

Inhibitory Potential of MBT-0206 and Taxol[®] against the highly drug-resistant murine colon carcinoma line Colon-26_{MBT}

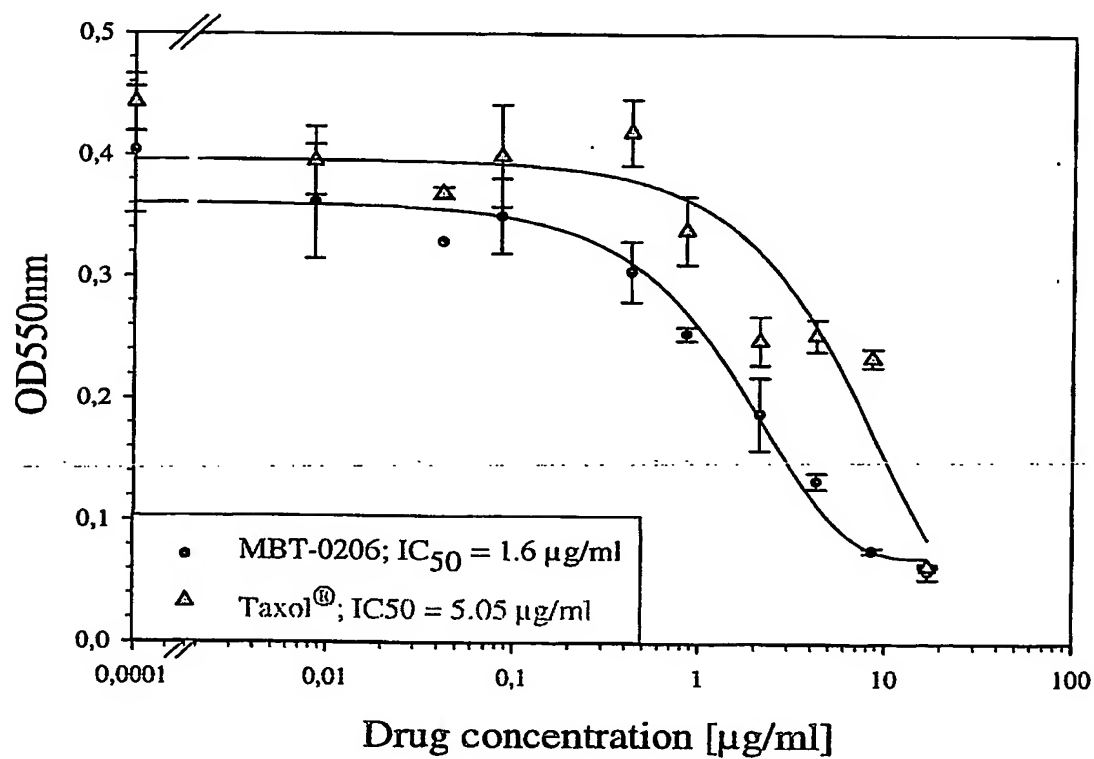


Figure 7

Inhibitory Potential of MBT-0206 and Taxol[®] against the parental drug-sensitive murine colon carcinoma line Colon-26

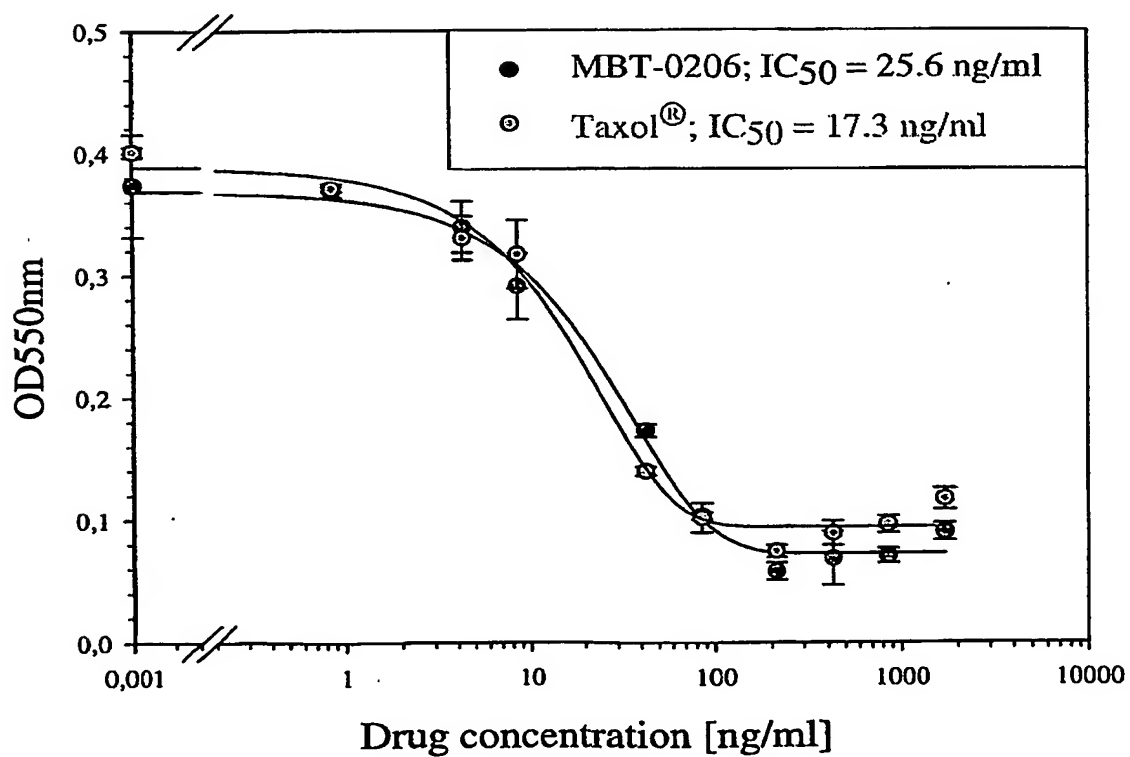
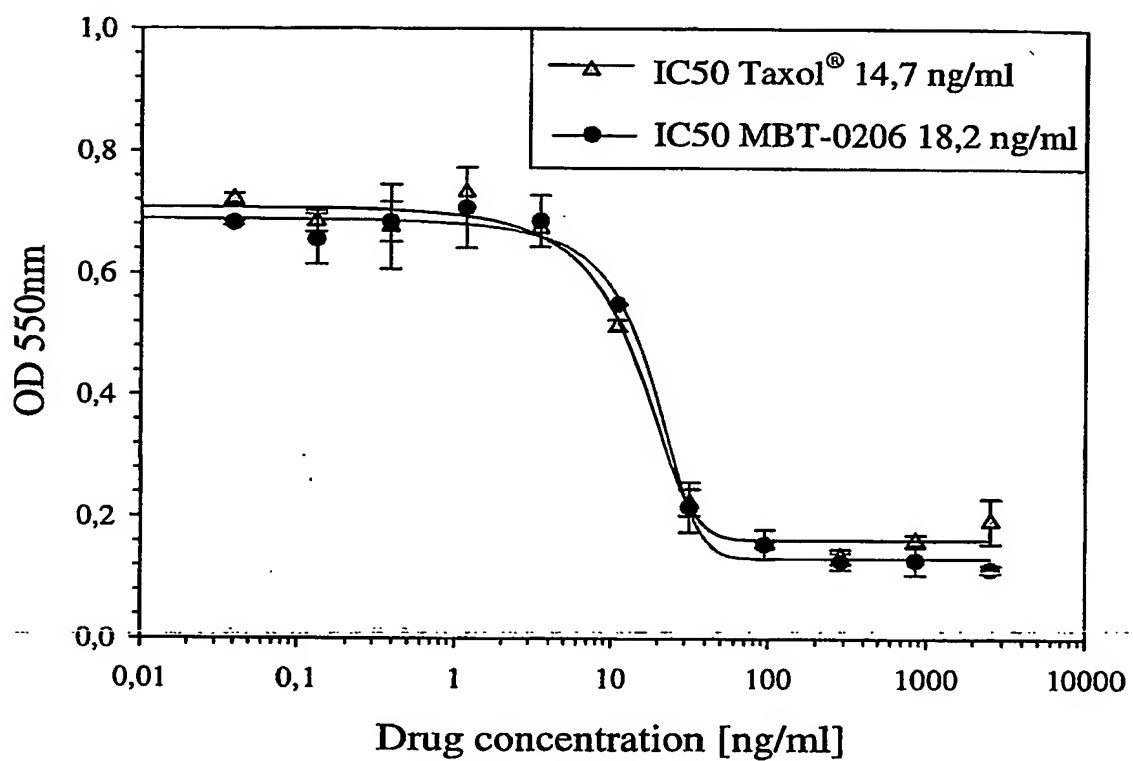


Figure 8

Inhibitory Potential of MBT-0206 and Taxol[®] against the drug-sensitive human endothelial line EA.hy926



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